

## APPENDIX I: Protocol for Topical Antibiotic Treatment

1. Create a treatment paste using powdered amoxicillin (example: Phytotechnology amoxicillin trihydrate, purity >95%) and Ocean Alchemist / Core Rx Base 2b.
  - a. Amoxicillin and Base2b should be refrigerated before use to increase shelf life.
  - b. Once amoxicillin and Base2b are mixed, degradation of amoxicillin occurs at approximately 1-2% per day. Ingredients should not be mixed more than a few days in advance of field application.
  - c. Take appropriate precautions for working with chemicals/pharmaceuticals.
2. Mix powdered amoxicillin into the Base in a 1:8 by weight ratio. Mixing can be done by hand using a metal spatula or butter knife in a large pot.
  - a. For a single small coral (e.g. in a nursery or a single target), 2.5 g amoxi + 20 g of Base is appropriate. For these smaller amounts, a balance is advised to weigh out the correct ratio.
  - b. For larger applications, jars of Base2b come in 400g amounts, so two jars can be mixed with one jar of 100g amoxicillin and stirred on the boat. For field treatments at a high-density site, a single experienced diver (~ 6 hours of bottom time) will average ~50 g amoxi + 400 g of Base2b.
3. Pack the mixture into the back of syringes for application using a small spatula or butter knife.
  - a. 60cc syringes are recommended for ease of application over multiple corals. Syringes can be reused
  - b. Catheter (tapered) syringes are recommended as they can be cut to increase tip diameter if application is difficult.
  - c. Syringes are positively buoyant. Sticking a lump of modeling clay onto each syringe is recommended to provide weight and prevent syringe loss.
4. At the SCTLD lesion, use the syringe to apply the treatment mixture over the lesion margin. Use a finger to press the product into the margin area. The treatment will be ~1 cm wide, with approximately half of that anchoring onto recently dead skeleton and the other half overlaying the live tissue. It adheres better to the skeleton than to the tissue, and should be pressed with moderate force for adherence. Small pieces may detach during application, but can generally be caught and remolded into to the application.
  - a. Compound adheres to nitrile gloves and neoprene gloves, which are not recommended. Other glove materials may be effective.
5. Alternative or additional intervention can be accomplished by creating and applying the compound to a firebreak about 5 cm away from the disease margin. An underwater angle grinder provides a rapid and clean trench, but this can also be accomplished with a hammer/chisel. Use the syringe to squeeze the amoxicillin mixture into the resulting trench. This provides a moderate increase in effectiveness but also substantially increases treatment time.
6. In rare instances, the treatment mixture will not adhere. In such cases, modeling clay can be used to strategically anchor the Base2b into place. Do not cover the entire treatment, but rather use small pieces of clay to weight or bridge the treatment into place.

Product	Weight	Price	Notes	Weblink for products that have been used in past efforts
Antibiotic (Amoxicillin)	25g	\$55.95		<a href="https://phytotechlab.com/amoxicillin.html">https://phytotechlab.com/amoxicillin.html</a> . Contact company directly for 100g jars and bulk discount. Veterinary/ranching alternatives may be cheaper, but effectiveness has not been tested.
Base 2b	400g	\$50.00	This amount will fill ~7 60cc syringes	Contact Ocean Alchemists. oceanalchemists@gmail.com
Catheter Syringe	10 syringes	\$10.99		<a href="https://www.amazon.com/Catheter-Syringe-Syringes-Care-Touch/dp/B01M1R392V/ref=sr_1_1_sspa?ie=UTF8&amp;qid=1537552151&amp;sr=8-1-spons&amp;keywords=catheter+syringe&amp;pssc=1">https://www.amazon.com/Catheter-Syringe-Syringes-Care-Touch/dp/B01M1R392V/ref=sr_1_1_sspa?ie=UTF8&amp;qid=1537552151&amp;sr=8-1-spons&amp;keywords=catheter+syringe&amp;pssc=1</a>
Modeling clay	2 lb	\$5.94		<a href="https://www.amazon.com/Sargent-Art-Plastilina-Modeling-2-Pound/dp/B00FR7TQOM/ref=sr_1_16?dchild=1&amp;keywords=modeling+clay&amp;qid=1591715628&amp;sr=8-16">https://www.amazon.com/Sargent-Art-Plastilina-Modeling-2-Pound/dp/B00FR7TQOM/ref=sr_1_16?dchild=1&amp;keywords=modeling+clay&amp;qid=1591715628&amp;sr=8-16</a>